U.S. App. No.: 09/826,206 Attorney Docket No: 030918 / NHN.0011.US01 John D'Elia

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The following Listing of the Claims will replace all prior versions and all prior listings of the claims in the present application:

Listing of The Claims:

(Previously presented) An isolated or purified nucleic acid molecule 1. comprising a Ketogulonigenium plasmid replicon as shown in SEQ ID NO: 1.

2-4. (Canceled)

- 5. (Previously presented) The nucleic acid molecule of claim 1, further comprising a replicon functional in E. coli.
- 6. (Previously presented) The nucleic acid molecule of claim 1, further comprising a replicon functional in an organism selected from the genera consisting of Acetobacter, Corynebacterium, Bacillus, Rhodobacter, Paracoccus, Roseobacter, Pseudomonas, Pseudogluconobacter, Gluconobacter, Serratia, Mycobacterium, and Streptomyces.
- 7. (Previously presented) The nucleic acid molecule of claim 1, further comprising a mob site.
- (Previously presented) The nucleic acid molecule of claim 7, wherein said 8. mob site comprises a mob gene and an oriT from a conjugation plasmid.
- 9. (Original) The nucleic acid molecule of claim 8, wherein said conjugation plasmid is selected from plasmids which are included within the incompatibility groups consisting of IncP, IncQ, IncC, IncB, IncF, IncG, IncI, IncK, IncM, IncN, IncPa, IncPb, IncW, IncX, and IncZ.
- 10. (Previously presented) The nucleic acid molecule of claim 1, further comprising a temperature-sensitive replicon.

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11. (Previously presented) The nucleic acid molecule of claim 1, further comprising at least one marker gene.

- (Original) The nucleic acid molecule of claim 11, wherein said marker 12. gene comprises a nucleotide sequence operative to direct synthesis of a protein conferring antibiotic resistance in a host cell population.
- 13. (Original) The nucleic acid molecule of claim 12, wherein said antibiotic is selected from the group comprising ampicillin, chloramphenicol, erythromycin, kanamycin, spectinomycin, streptomycin and tetracycline.
- 14. (Original) The nucleic acid molecule of claim 1, comprising at least one further nucleic acid sequence, wherein said further nucleic acid sequence is selected from the group consisting of a polylinker insert, an expression control sequence, a cos site, a terminator sequence, a ribosome binding site, a DNA sequence encoding a signal peptide, a DNA sequence encoding a polypeptide and a DNA sequence encoding a polypeptide containing one or more signal peptides.

15-16. (Cancelled)

- (Original) The nucleic acid molecule of claim 14, further comprising a His-17. Tag sequence.
- 18. (Previously presented) The nucleic acid molecule of claim 14, further comprising a nucleic acid sequence encoding a polypeptide sequence not expressed natively in Ketogulonigenium.
- (Previously presented) The nucleic acid molecule of claim 14, wherein 19. said further nucleic acid sequence is said cos site.
- 20. (Original) The nucleic acid molecule of claim 1, further comprising a DNA sequence from an E. coli-derived plasmid.

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21. (Previously presented) The nucleic acid molecule of claim 20, wherein said *E. coli*-derived plasmid is selected from the group consisting of pET, pUC18, and pUC19.

- 22. (Original) The nucleic acid molecule of claim 1, further comprising a reporter gene.
- 23. (Previously presented) The nucleic acid molecule of claim 22, wherein said reporter gene encodes a protein selected from the group consisting of β-galactosidase, β-glucuronidase, luciferase, green fluorescent protein α-amylase, and uroporphyrinogen III methyltransferase (cobA) from *Propionibacterium freudenreichii*.
- 24. (Previously presented) The nucleic acid molecule of claim 1, wherein said nucleic acid molecule autonomously replicates in *Ketogulonigenium* and in at least one organism selected from the genera consisting of *Acetobacter, Corynebacterium, Bacillus, Rhodobacter, Paracoccus, Roseobacter, Pseudomonas, Pseudogluconobacter, Gluconobacter, Serratia, Mycobacterium*, and *Streptomyces*.
- 25. (Original) A transformed *Escherichia coli* cell comprising the nucleic acid molecule of claim 1.
- 26. (Original) A transformed *Ketogulonigenium* cell comprising the nucleic acid molecule of claim 1.
- 27. (Previously presented) A method for producing a polypeptide, comprising culturing a host cell comprising an isolated or purified nucleic acid molecule comprising a polynucleotide having the nucleotide sequence of a *Ketogulonigenium* plasmid replicon as shown in SEQ ID NO:1, and comprising at least one further nucleic acid sequence, wherein said further nucleic acid sequence is selected from the group consisting of a polylinker insert, an expression control sequence, a cos site, a terminator sequence, a ribosome binding site, a DNA sequence encoding a signal peptide, a DNA sequence encoding a polypeptide

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containing one or more signal peptides, under conditions such that said polypeptide is expressed, and recovering said polypeptide.

- 28. (Previously presented) A method of transforming a host cell with a nucleic acid comprising:
 - (a) obtaining a host cell;
- (b) transforming the host cell of (a) with an isolated or purified nucleic acid molecule comprising a polynucleotide having the nucleotide sequence of a *Ketogulonigenium* plasmid replicon as shown in SEQ ID NO:1, comprising at least one further nucleic acid sequence, wherein said further nucleic acid sequence is selected from the group consisting of a polylinker insert, an expression control sequence, a cos site, a terminator sequence, a ribosome binding site, a DNA sequence encoding a signal peptide, a DNA sequence encoding a polypeptide and a DNA sequence encoding a polypeptide containing one or more signal peptides; and
 - (c) obtaining a stably transformed host cell.
- 29. (Original) The method of claim 28, wherein said transformation comprises conjugation.
- 30. (Original) The method of claim 28, wherein said transformation comprises electroporation.
- 31. (Previously presented) The nucleic acid molecule of claim 14, wherein said further nucleic acid sequence is said expression control sequence.
- 32. (Previously presented) The nucleic acid molecule of claim 31, wherein said expression control sequence comprises an *E. coli*-derived promoter.
- 33. (Previously presented) The nucleic acid molecule of claim 31, wherein said expression control sequence comprises a *Ketogulonigenium*-derived promoter.
- 34. (Previously presented) The nucleic acid molecule of claim 14, wherein said further nucleic acid sequence is said polylinker insert.

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35. (Previously presented) The nucleic acid molecule of claim 14, wherein said further nucleic acid sequence is said terminator sequence.

- 36. (Previously presented) The nucleic acid molecule of claim 14, wherein said further nucleic acid sequence is said ribosome binding site.
- 37. (Previously presented) The nucleic acid molecule of claim 14, wherein said further nucleic acid sequence is said DNA sequence encoding a signal peptide.
- 38. (Previously presented) The nucleic acid molecule of claim 14, wherein said further nucleic acid sequence is said DNA sequence encoding a polypeptide.
- 39. (Previously presented) The nucleic acid molecule of claim 14, wherein said further nucleic acid sequence is said DNA sequence encoding a polypeptide containing one or more signal peptides.

40-43. (Canceled)

- 44. (Previously presented) The nucleic acid molecule of claim 1, wherein the nucleic acid sequence of the replicon is at least 96% identical over 2100 consecutive base pairs to SEQ ID NO:1.
- 45. (Previously presented) The nucleic acid molecule of claim 1, wherein the nucleic acid sequence of the replicon is at least 97% identical over 2100 consecutive base pairs to SEQ ID NO:1.
- 46. (Previously presented) The nucleic acid molecule of claim 1, wherein the nucleic acid sequence of the replicon is at least 98% identical over 2100 consecutive base pairs to SEQ ID NO:1.
- 47. (Previously presented) The nucleic acid molecule of claim 1, wherein the nucleic acid sequence of the replicon is at least 99% identical over 2100 consecutive base pairs to SEQ ID NO:1.

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48. (Currently Amended) An isolated <u>polynucleotide</u> nucleic acid molecule comprising a *Ketogulonigenium* replicon, wherein the <u>polynucleotide</u> nucleic acid comprises a sequence at least 95% identical to nucleotides 2955-49602960 of SEQ ID NO:2, immediately followed by a sequence at least 95% identical to nucleotides 2955-49602960 of SEQ ID NO:2.

- 49. (Previously presented) A shuttle vector that has at least 95% sequence identity to SEQ ID NO:3.
- 50. (Previously presented) The shuttle vector of claim 49, wherein the shuttle vector has at least 97% sequence identity to SEQ ID NO:3.
- 51. (Previously presented) The shuttle vector of claim 49, wherein the shuttle vector has at least 99% sequence identity to SEQ ID NO:3.
- 52. (Previously presented) A shuttle vector comprising the nucleic acid sequence of SEQ ID NO:3.
- 53. (Previously presented) The nucleic acid molecule of claim 5, wherein said nucleic acid molecule autonomously replicates in *Ketogulonigenium* and *E. coli*.
- 54. (Previously presented) The nucleic acid molecule of claim 44, wherein said nucleic acid molecule autonomously replicates in *Ketogulonigenium* and *E. coli*.
- 55. (Previously presented) The nucleic acid molecule of claim 48, wherein said nucleic acid molecule autonomously replicates in *Ketogulonigenium* and *E. coli*.
- 56. (Previously presented) The nucleic acid molecule of claim 49, wherein said nucleic acid molecule autonomously replicates in *Ketogulonigenium* and *E. coli*.